

High Rate of Human Bocavirus and Adenovirus Coinfection in Hospitalized Israeli Children[▽]

Musa Y. Hindiye^{1,*}, Nathan Keller,² Michal Mandelboim,¹ Daniela Ram,¹ Jana Rubinov,¹
Liora Regev,¹ Virginia Levy,¹ Sara Orzitzer,¹ Hilda Shaharabani,¹ Roberto Azar,¹
Ella Mendelson,^{1,3,†} and Zehava Grossman^{1,†}

Central Virology Laboratory, Public Health Services, Ministry of Health, Sheba Medical Center, Tel-Hashomer, Israel¹;
Department of Clinical Microbiology, Sheba Medical Center, Tel-Hashomer, Israel²; and Faculty of Life Sciences,
Bar Ilan University, Ramat-Gan, Israel³

Received 14 August 2007/Returned for modification 24 September 2007/Accepted 17 October 2007

We investigated coinfection of human bocavirus (HBoV) and other respiratory viruses in hospitalized children by real-time PCR. A high rate (69.2%) of adenovirus infection was found among children infected with HBoV. Such high rates of HBoV-adenovirus coinfection have not been previously reported, underscoring the need to investigate the contribution of HBoV in patient clinical presentations.

The discovery in 2005 of human bocavirus (HBoV) among Swedish children by Allander et al. (1) added a new member to the list of viruses that can cause respiratory tract infections. HBoV belongs to the *Parvovirinae* subfamily of the *Parvoviridae* family (1). HBoV is a nonenveloped, single-stranded DNA virus with a 5.2-kb-long genome. Based on DNA sequencing, two groups of HBoV were identified, Stockholm 1 (st 1) and Stockholm 2 (st 2) (1). Since its discovery, HBoV has been found in several countries (2, 6, 8, 14–23, 25).

Allander et al. (1) initially detected HBoV DNA in 3.1% of Swedish children and infants presenting with variable degrees of respiratory distress and fever. Moreover, 17.6% of HBoV-positive patients were also positive for either adenovirus or respiratory syncytial virus (RSV) (1). Since then, the frequency of HBoV reported from several studies worldwide has ranged between 1.5% and 18.3%, and the rate of coinfection of HBoV and other respiratory viruses, particularly coinfection with rhinovirus, RSV, or parainfluenza virus, was as high as 91% (9). The highest coinfection rate of HBoV and adenovirus reported to date was 37.1% in Korea (5). The high coinfection rate with other viruses and the difficulties in culturing HBoV could be in part due to its dependence on other respiratory viruses for its replication. Such dependence would be reminiscent of the adenoassociated *Dependovirus* virus, which is dependent on adenovirus for its replication (4, 10, 24).

In this study we evaluated the rate of coinfection with HBoV and adenovirus in 231 Israeli children. These children were hospitalized between January 2006 and December 2006 with upper-respiratory-system infection, and adenovirus was on the list of viruses to be checked or the patients had clinical symptoms most closely resembling adenovirus infection. The ages of the patients ranged from a few days to less than 10 years. The male-to-female ratio was 1.4:1.0. The difference tended to be

larger for those diagnosed with HBoV infection or adenovirus infection (male-to-female ratio, 1.9 or 1.8, respectively).

Patient samples were obtained by well-trained health care providers and transported to the Israel Central Virology Laboratory in a timely manner. The samples included 97 (42%) nasal suction samples, 69 (30%) nose swab samples, 24 (10.4%) bronchoalveolar lavage (BAL) samples, 23 (10%) throat swab samples, 12 (5%) sputum samples, 3 (1.3%) lung biopsy samples, and 3 (1.3%) pleural fluid samples. All were examined for the presence of adenovirus. The presence of other viruses was tested according to the requests of physicians. HBoV was tested for retrospectively in all samples.

The samples were tested for the presence of adenovirus, HBoV, influenza A and B virus, and RSV A and B by real-time PCR using TaqMan chemistry as previously described (8, 11–13), while parainfluenza viruses 1, 2, and 3 were tested for by reverse transcription-PCR with gel detection as previously reported (7). Viral genomic DNA was extracted from patient samples by use of a QIAamp DNA blood Mini kit (Qiagen GmbH, Hilden, Germany), and viral genomic RNA was extracted using a High Pure viral RNA extraction kit (Roche Diagnostics GmbH, Mannheim, Germany).

Of the 231 samples analyzed, 76 (32.9%) were positive for adenovirus DNA and 26 (11.3%) were positive for HBoV. The high adenovirus positivity rate found (32.9% versus 13.9% for all other viruses together except HBoV; $P < 0.0001$ [t test]) likely reflects, in large part, preselection of patients suspected to be infected with this virus. The frequency of HBoV (11.3%) was higher than that found in Europe (25), equal to that reported from Korea, and less than that reported from Jordan (18.3%) (14). Eighteen (69.2%) of the 26 HBoV-positive samples were coinfecting with adenovirus, a result significantly above the frequency of adenovirus infection found in the non-HBoV-infected children (28.3%; $P < 0.001$ [t test]). Even more meaningful is the finding of a higher frequency of HBoV infection among adenovirus-infected children (23.7%) than in non-adenovirus-infected children (5.2%; $P < 0.0001$, [t test]). Such high levels of association between HBoV and adenovirus had not been reported previously. The difference could be

* Corresponding author. Mailing address: Central Virology Laboratory, Chaim Sheba Medical Center, Tel-Hashomer 52621, Israel. Phone: 972-3-530-2066. Fax: 972-3-530-2457. E-mail: hindiye@yahoo.com.

† E.M. and Z.G. contributed equally.

▽ Published ahead of print on 31 October 2007.

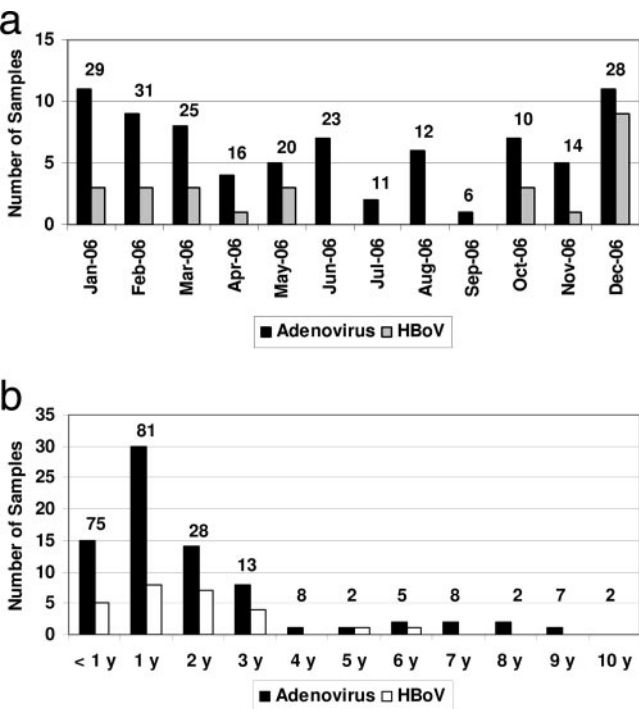


FIG. 1. (a) Histogram of the monthly distribution of clinical samples that were PCR positive for adenovirus and HBoV DNA among hospitalized Israeli patients during the year 2006. The number of patients tested in each age group is indicated on the top of each bar. (b) Age distribution of patients samples that were positive for adenovirus and HBoV during the year 2006. The number of patients tested in each age group is indicated on the top of each bar.

attributed in part to the use of highly sensitive molecular assays in the present study. Determining the adenovirus serotypes of 7 of 18 available coinfectd samples revealed the presence of three different serotypes (T1 [3 of 7], T2 [3 of 7], and T3 [1 of 7]). None of the serotyped samples showed coinfection by multiple adenoviruses. These were the most prevalent adenovirus serotypes found during the study period (each of them was found in 28.5% [10 of 35] of samples tested; the other serotypes were T5 [3 of 35] and T7 [2 of 35]). These results suggest that HBoV coinfection does not depend on the presence of a particular adenovirus serotype.

HBoV infections with other respiratory viruses have been previously reported to occur at rates in the range of 17% to 91% (1, 9). In particular, high (91%) rates of HBoV coinfections with rhinovirus, RSV, or parainfluenza virus have been previously reported in studies of hospitalized children less than 5 years of age with pneumonia (9). While rhinovirus infections were not investigated in our patient population, only two of the eight HboV-positive and adenovirus-negative samples were coinfectd with either RSV (1 of 14 [7.1%]) or parainfluenza virus 3 (1 of 10 [10%]). Thus, the overall rate of coinfection of HBoV with other evaluated respiratory viruses was 76.9%.

Interestingly, of the patient sample types evaluated, HBoV DNA was mostly detected in throat swab patient samples (6 of 23 [26.1%]) compared to BAL (3 of 24 [12.5%]), nose swab (7 of 62 [10.1%]), or nasal suction (9 of 88 [9.3%]) patient samples. On the other hand, adenovirus DNA was detected at similar rates (32% to 39%) in throat, BAL, and nasal suction patient samples. Adenovirus and HBoV were each detected in pleural fluid from a patient, while no viral DNA was detected

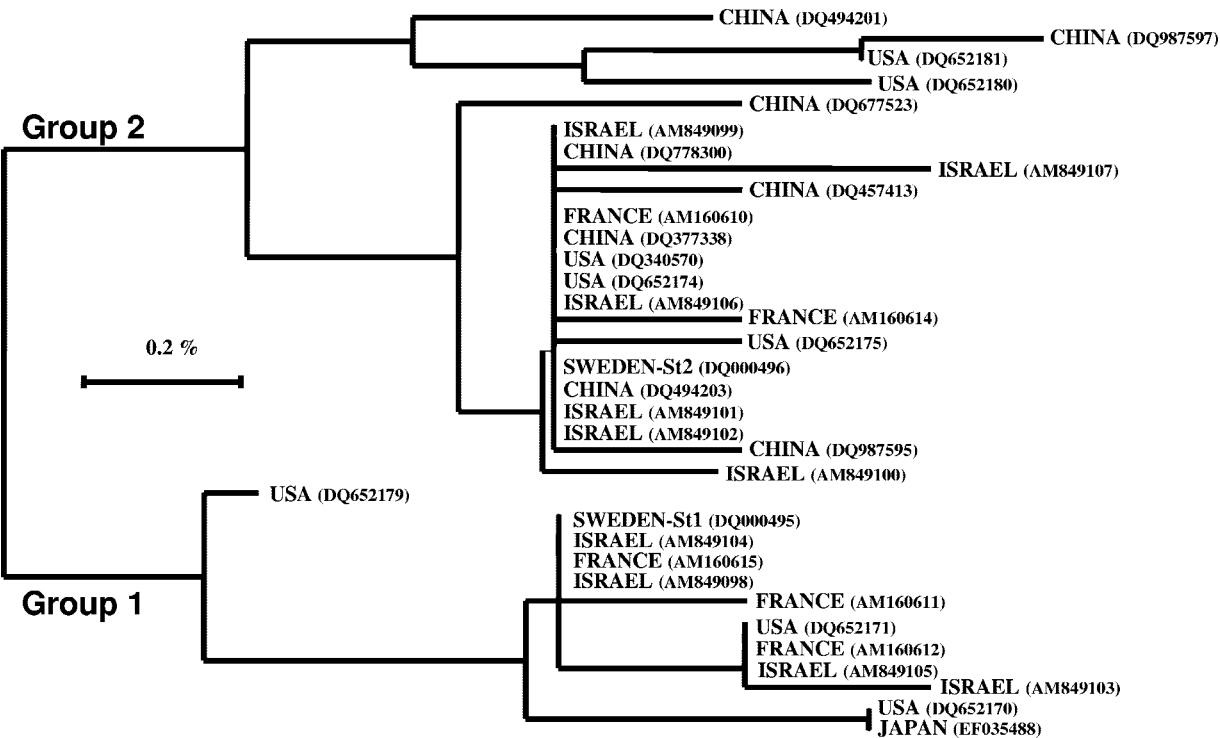


FIG. 2. Phylogenetic comparison of HBoV VP2 gene nucleotides detected during the year 2006 with sequences from EMBL and GenBank databases. The Clustal X nearest-neighbor joining method (1,000 bootstraps) was used to compare 417-nucleotide sequences encoding VP2 genes with sequences from the EMBL and GenBank databases.

in either sputum or lung biopsy samples. Coinfection of HBoV and adenovirus was most frequently detected in nasal suction samples (7 of 18 [38.9%]) followed by nose swab samples (5 of 18 [27.8%]) and throat swab samples (4 of 18 [22.2%]).

Stratifying analyzed patient samples by the month in which the infection occurred showed that adenovirus circulated throughout the year, with major activity during the respiratory illness season, in particular, during January and December 2006, while HBoV DNA was detected from January to May and then from September to December 2006 (Fig. 1a). The time distribution of HBoV-positive samples was similar to that reported from the United States, France, and Germany (8, 15, 25). Bastien et al. reported collection of HBoV-positive samples throughout 2003, with the exception of the month of August, from patients in the colder nation of Canada (3). In Israel, the rate of coinfection by HBoV and adenovirus was highest during December 2006 (7 of 18 [38.9%]).

Adenovirus was detected in Israeli children up to 10 years of age, in particular, in those under the age of 3 (Fig. 1b). Of the patients positive for adenovirus, 15 (6.5%) were younger than 1 year, 30 (13%) were 1 year old, 14 (6%) were 2 years old, and 8 (3.5%) were 3 years old. HBoV DNA was detected in children less than 6 years old, mainly those under 3 years old (Fig. 1b). HBoV was detected in five (2.2%) patients who were under 1 year of age, eight (3.5%) who were 1 year old, seven (3.0%) who were 2 years old, and four (1.7%) who were 3 years old. Coinfection with adenovirus and HBoV was highest in 1-year-old patients (6 of 18 [33.3%]) but was also frequent in patients under 1 year of age (4 of 18 [22%]) and in those who were 2 years old (4 of 18 [22%]). The age distribution of HBoV-infected children was similar to that reported from other countries (17, 25).

Phylogenetic analysis was performed on a part of the VP2 genes of 10 HBoV-positive samples detected between January and December 2006. Briefly, primer sequences BocaSEQ3 (forward) and BocaSEQ4 (reverse) derived from the VP2 were used for this analysis of HBoV-positive patient samples and to confirm HBoV-positive patient samples by use of the NP1 gene as described by Foulongne et al. (8). The 514-bp PCR products were gel purified using Qiagen QIAquick gel extraction (Qiagen GmbH, D-40724 Hilden, Germany) and sequenced using an ABI Prism dye Deoxy Terminator cycle sequencing kit (Applied Biosystems, Foster City, CA). Reaction mixtures were analyzed on Applied Biosystems model 373 DNA automatic sequencing systems. The Sequencher program (Gencodes Corporation, Ann Arbor, MI) was used to compare 417-bp nucleotide sequences. A phylogenetic tree was prepared by nearest-neighbor analysis using Clustal X with 1,000 bootstraps, and trees were visualized using TreeView or NJ plot software. Sequences from all 10 isolates (EMBL accession numbers AM849098 to AM849107) had high (99 to 100%) homology to HBoV sequences in the EMBL, GenBank, and DDBJ databases (Fig. 2). The two main groups, Stockholm 1 (GenBank accession number DQ000495) and Stockholm 2 (GenBank accession number DQ000496), which had been reported in several areas of the world, also circulated in Israel during 2006, as illustrated with 10 randomly selected HBoV-positive samples from Israeli patients (Fig. 2).

Our study showed that both HBoV groups circulated in Israeli children less than 10 years of age, in particular in those

under 3, during the year 2006. The frequency in our selected patient population was 11.3%, at the upper end of what had been reported worldwide. This high HBoV frequency could be due to the evaluation of patients clinically suspected of having adenovirus. The high rate of coinfection of adenovirus and HBoV in hospitalized Israeli children exemplifies the importance of examining the role that HBoV may play in the clinical presentations of the hospitalized patients diagnosed with adenovirus infection. In addition, it would be worthwhile to investigate whether HBoV replication is dependent in part on the presence of adenovirus or of any other human respiratory virus.

Nucleotide sequence accession numbers. The HBoV sequences have been assigned EMBL accession numbers AM849098 to AM849107.

We thank Vincent Foulongne from the Department of Virology, University Hospital of Montpellier, France, for providing us with the HBoV-positive control.

REFERENCES

1. Allander, T., M. T. Tammi, M. Eriksson, A. Bjerkner, A. Tiveljung-Lindell, and B. Andersson. 2005. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc. Natl. Acad. Sci. USA* **102**:12891–12896.
2. Bastien, N., K. Brandt, K. Dust, D. Ward, and Y. Li. 2006. Human bocavirus infection, Canada. *Emerg. Infect. Dis.* **12**:848–850.
3. Bastien, N., N. Chui, J. L. Robinson, B. E. Lee, K. Dust, L. Hart, and Y. Li. 2007. Detection of human bocavirus in Canadian children in a 1-year study. *J. Clin. Microbiol.* **45**:610–613.
4. Berns, K. I., and W. W. Hauswirth. 1979. Adeno-associated viruses. *Adv. Virus Res.* **25**:407–449.
5. Choi, E. H., H. J. Lee, S. J. Kim, B. W. Eun, N. H. Kim, J. A. Lee, J. H. Lee, E. K. Song, S. H. Kim, J. Y. Park, and J. Y. Sung. 2006. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000–2005. *Clin. Infect. Dis.* **43**:585–592.
6. Chung, J. Y., T. H. Han, C. K. Kim, and S. W. Kim. 2006. Bocavirus infection in hospitalized children, South Korea. *Emerg. Infect. Dis.* **12**:1254–1256.
7. Echevarría, J. E., D. D. Erdman, E. M. Swierkosz, B. P. Holloway, and L. J. Anderson. 1998. Simultaneous detection and identification of human parainfluenza viruses 1, 2, and 3 from clinical samples by multiplex PCR. *J. Clin. Microbiol.* **36**:1388–1391.
8. Foulongne, V., Y. Olejnik, V. Perez, S. Elaerts, M. Rodiere, and M. Segondy. 2006. Human bocavirus in French children. *Emerg. Infect. Dis.* **12**:1251–1253.
9. Fry, A. M., X. Lu, M. Chittaganpitch, T. Peret, J. Fischer, S. F. Dowell, L. J. Anderson, D. Erdman, and S. J. Olsen. 2007. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J. Infect. Dis.* **195**:1038–1045.
10. Handa, H., and B. J. Carter. 1979. Adeno-associated virus DNA replication complexes in herpes simplex virus or adenovirus-infected cells. *J. Biol. Chem.* **254**:6603–6610.
11. Heim, A., C. Ebnet, G. Harste, and P. Pring-Akerblom. 2003. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J. Med. Virol.* **70**:228–239.
12. Hindiyeh, M., V. Levy, R. Azar, N. Varsano, L. Regev, Y. Shalev, Z. Grossman, and E. Mendelson. 2005. Evaluation of a multiplex real-time reverse transcriptase PCR assay for detection and differentiation of influenza viruses A and B during the 2001–2002 influenza season in Israel. *J. Clin. Microbiol.* **43**:589–595.
13. Hu, A., M. Colella, J. S. Tam, R. Rappaport, and S. M. Cheng. 2003. Simultaneous detection, subgrouping, and quantitation of respiratory syncytial virus A and B by real-time PCR. *J. Clin. Microbiol.* **41**:149–154.
14. Kaplan, N. M., W. Dove, A. F. Abu-Zeid, H. E. Shamoon, S. A. Abd-ElDayem, and C. A. Hart. 2006. Human bocavirus infection among children, Jordan. *Emerg. Infect. Dis.* **12**:1418–1420.
15. Kesebir, D., M. Vazquez, C. Weibel, E. D. Shapiro, D. Ferguson, M. L. Landry, and J. S. Kahn. 2006. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J. Infect. Dis.* **194**:1276–1282.
16. Lin, F., A. Zeng, N. Yang, H. Lin, E. Yang, S. Wang, D. Pintel, and J. Qiu. 2007. Quantification of human bocavirus in lower respiratory tract infections in China. *Infect. Agents Cancer* **2**:3.

17. **Ma, X., R. Endo, N. Ishiguro, T. Ebihara, H. Ishiko, T. Ariga, and H. Kikuta.** 2006. Detection of human bocavirus in Japanese children with lower respiratory tract infections. *J. Clin. Microbiol.* **44**:1132–1134.
18. **Manning, A., V. Russell, K. Eastick, G. H. Leadbetter, N. Hallam, K. Templeton, and P. Simmonds.** 2006. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. *J. Infect. Dis.* **194**:1283–1290.
19. **Monteny, M., H. G. Niesters, H. A. Moll, and M. Y. Berger.** 2007. Human bocavirus in febrile children, The Netherlands. *Emerg. Infect. Dis.* **13**:180–182.
20. **Naghipour, M., L. E. Cuevas, T. Bakhshinejad, W. Dove, and C. A. Hart.** 2007. Human bocavirus in Iranian children with acute respiratory infections. *J. Med. Virol.* **79**:539–543.
21. **Regamey, N., U. Frey, C. Deffernez, P. Latzin, and L. Kaiser.** 2007. Isolation of human bocavirus from Swiss infants with respiratory infections. *Pediatr. Infect. Dis. J.* **26**:177–179.
22. **Sloots, T. P., P. McErlean, D. J. Speicher, K. E. Arden, M. D. Nissen, and I. M. Mackay.** 2006. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J. Clin. Virol.* **35**:99–102.
23. **Smuts, H., and D. Hardie.** 2006. Human bocavirus in hospitalized children, South Africa. *Emerg. Infect. Dis.* **12**:1457–1458.
24. **Timpe, J. M., K. C. Verrill, and J. P. Trempe.** 2006. Effects of adeno-associated virus on adenovirus replication and gene expression during coinfection. *J. Virol.* **80**:7807–7815.
25. **Weissbrich, B., F. Neske, J. Schubert, F. Tollmann, K. Blath, K. Blessing, and H. W. Kreth.** 2006. Frequent detection of bocavirus DNA in German children with respiratory tract infections. *BMC Infect. Dis.* **6**:109.